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ARTIFICIAL SWEETENERS AND HUMAN BLADDER CANCER

Preliminary Results

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Summary 3010 patients with cancer of the urinary bladder and 5783 controls drawn from the general population of ten geographic areas of the U.S.A. were interviewed. Subjects who reported ever having used artificial sweeteners or artificially sweetened foods or beverages showed no elevation in risk. However, positive associations between various measures of use of artificial sweeteners and risk of bladder cancer were seen in several subgroups. Inconsistencies in the data suggest that the positive associations may be due to chance, but it is noteworthy that the subgroups were those chosen, a priori, to test hypotheses derived from laboratory experiments.

Introduction

IN 1977 a case-control interview study[1] revealed that men who used artificial sweeteners (AS) had a 60% increase in their risk of bladder cancer, with evidence of a dose-response relationship. However, women had a non-significant decrease in risk. A subsequent unpublished report from an ongoing case-control study also disclosed an excess risk of bladder cancer among male users of AS but not among female users,[2] but a later report from the same study found no excess.[3] Other case-control studies have not revealed an association for either sex.[4-7] Experimental evidence has shown that saccharin, a combination of cyclamate and saccharin, and a metabolite of cyclamate can each cause bladder tumours in rats if the animals are given high doses.[8-11] The results for saccharin were most obvious if the exposure began in utero. Laboratory studies have also shown that saccharin promotes the carcinogenic effects of other agents.[12,13]

We conducted a large, population-based case-control interview study for two purposes: to resolve some of the apparent conflicts in the findings of the epidemiological studies and to look for evidence of either of the two biological mechanisms suggested for saccharin by laboratory data—weak carcinogenesis when given by itself, and potentiation of carcinogenesis when given with other carcinogens. We concluded that a large study was needed in order to provide adequate numbers of subjects in separate subgroups where these two mechanisms might be manifest. In particular, we wished to examine the subjects who were at the highest level of exposure. We also wished to examine the subjects at low background risk of bladder cancer among whom a small addition to risk might be most discernible. Third, we wished to examine the subjects exposed to known bladder carcinogens before they were exposed to AS, among whom evidence of promotion might be detectable.

Methods

Cases comprised all residents of designated counties in the metropolitan areas of Atlanta, Detroit, New Orleans, San Francisco, and Seattle and in the states of Connecticut, Iowa, New Jersey, New Mexico, and Utah, aged 21–84, who were newly diagnosed with a histologically confirmed carcinoma of the urinary bladder (or papilloma not specified as benign) during a one-year period beginning in December, 1977. Cases with previous lower-urinary-tract cancers were excluded. The cases were found through the Surveillance, Epidemiology and End Results Network and the New Jersey Cancer Registry. Controls were an age and sex stratified random sample of the general populations of the ten geographic areas, frequency-matched at a 2:1 ratio of controls to cases.

Controls aged 65–84 were randomly sampled from the files of the Health Care Financing Administration, which enumerated an estimated 98% of individuals over age 65 in the U.S.A. Controls aged 21–64 were selected in a three-stage process: telephone numbers were chosen at random from all residential telephones in the ten geographic areas;[14] an interviewer called each number and recorded the age and sex of each household member aged 21–64; a stratified random sample was selected from the household censuses.

Personal interviews were conducted in the subjects' homes. Questionnaire items included detailed histories of use of AS in three forms (as a table-top sweetener, in diet drinks, in diet

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foods) as well as tobacco use, occupation, residence, source of water, coffee use, hair-dye use, and illnesses.

In the analysis, the unexposed groups included only subjects who were never exposed to any form of AS. In tables referring to only one form of AS, the exposed groups included only those who used that form (whether or not exposed to other forms). Since only 1% of the subjects used diet food but not diet drinks or table-top AS, data are not presented for diet foods in detail.

The measure of strength of association used is the maximum likelihood estimate of relative risk (RR) derived from the odds ratio.[15] Where noted, analyses were controlled for other potentially confounding variables by multiple contingency table analysis[16] or logistic regression.[17] One-tailed tests for statistical significance were used—the Mantel-Haenszel summary chi-squared[18] and the Mantel extension of the Mantel-Haenszel test, a test for linear trend.[19]

During the study, 4045 eligible cases were identified, of whom 1% had papillomas and 99% had carcinomas. From the files of the Health Care Financing Administration, we drew 4058 controls aged 65–84. Of the 25 826 residential telephone numbers we chose at random, 88% yielded household censuses. We could not attempt to interview subjects from households that gave no census. Nor could we interview those who had died (7% of cases, 1% of controls) or those severely disabled (7% of cases, 3% of controls). Among those approached for an interview, cooperation rates were 87% for all cases, 85% for controls aged 21–64, 87% for controls aged 65–84, 86% for subjects overall. If the numbers of identified cases and controls are combined with the estimated number of controls that would have been eligible had telephone censuses been obtained, the total is 11 430, of whom we interviewed 8793.

Results

75% of the cases (and controls) were male and the median age was 67. Compared with the control series, the case series included more White subjects, more cigarette-smokers, and more workers exposed to dye, rubber, leather, ink, or paint. These patterns replicated those of previous studies.[20]

Men who had ever used AS showed a relative risk of 0·99 and the corresponding women showed a relative risk of 1·07, after adjustment for the identified risk factors in the total group—race, cigarette-smoking, coffee-drinking, and chemical exposures at work (table I). Relative risks for use of each major form of AS did not differ appreciably from 1·00. Additional control (for age, sex, history of diabetes, geographic area, and education) did not alter the estimates of relative risk.

Users of table-top AS or diet drink were classified according to their usual level of intake (table II). Although males with heavier usual consumption of diet drinks showed elevated risks, there was no consistent gradation of risk with increased use of diet drinks or table-top AS. Among females, the risks were elevated with heavier use, and the trend for table-top AS was statistically significant. However, the relative risks were below 1·0 in some categories and the patterns were variable. Combined consumption of diet drinks and table-top AS was also considered, with categories defined to reflect the fact that one average serving of diet drink contains 2–3 times as much AS as one average use of table-top AS (table III). Subjects who used both forms, at least one of them heavily, showed an increased risk. Logistic regression analysis controlling for sex, age, race, smoking, occupational exposures, region, and education yielded a relative risk of 1·45 for those who used at least three servings of table-top AS and at least two diet drinks daily or who used at least some diet drinks and at least six servings of table-top AS (the three categories of greatest use in table III). (The 95% confidence interval was from 1·00 to 2·10.) For males, the relative risk was 1·47; for females, 1·41.

When subjects were categorised according to the duration of exposure or according to the years since the first exposure, no consistent patterns emerged for either sex. In fact, for males and females, the lowest risk was seen in the subjects with longest use. An analysis of esti-

TABLE I—HISTORY OF USE OF ARTIFICIAL SWEETENERS, BY SEX

	Cases	Controls	R R*	95% Confidence limits
Males				
Never used AS	1349	2554	1·00	
Ever used diet drink	607	1204	0·95	(0·84, 1·07)
Ever used table-top	592	1066	1·04	(0·92, 1·18)
Ever used diet food	240	442	1·02	(0·85, 1·22)
Ever used any form	909	1723	0·99	(0·89, 1·10)
Females				
Never used AS	358	767	1·00	
Ever used diet drink	262	504	1·02	(0·83, 1·25)
Ever used table-top	236	474	1·04	(0·84, 1·28)
Ever used diet food	130	239	1·13	(0·87, 1·47)
Ever used any form	384	732	1·07	(0·89, 1·29)
Both sexes				
Never used AS	1707	3321	1·00	
Ever used diet drink	869	1708	0·97	(0·87, 1·07)
Ever used table-top	828	1540	1·04	(0·93, 1·16)
Ever used diet food	370	681	1·05	(0·91, 1·22)
Ever used any form	1293	2455	1·01	(0·92, 1·11)

* Relative risk adjusted for race, cigarette smoking, coffee drinking, and occupational exposure.

TABLE II—AVERAGE DAILY USE OF TABLE-TOP SWEETENERS, AND OF DIET DRINK

—	Males			Females		
	Cases	Controls	R R*	Cases	Controls	R R*
Never used AS	1349	2554	1·00	358	767	1·00
<1 use table-top	109	190	1·09	39	113	0·73
1–1·9 uses table-top	105	229	0·88	56	96	1·28
2–3·9 uses table-top	164	299	1·08	72	110	1·42
4–5·9 uses table-top	62	118	0·97	22	45	0·99
⩾6 uses table-top	39	59	1·05	16	20	1·36
	(χ^2=0·181; p=0·43)			(χ^2=1·938; p=0·03)		
<1 serving diet drink	349	723	0·93	146	294	1·01
1–1·9 servings diet drink	107	207	0·93	44	108	0·83
2–2·9 servings diet drink	48	63	1·44	24	29	1·72
⩾3 servings diet drink	25	41	1·01	15	20	1·37
(χ^2=0·352; p=0·36)	(χ^2=0·352; p=0·36)			(χ^2=0·942; p=0·17)		

* Relative risk adjusted for age, race, and cigarette smoking. p values are based on one-tailed tests.

TABLE III—AVERAGE NUMBER OF DAILY USES OF TABLE-TOP SWEETENERS BY AVERAGE NUMBER OF DAILY SERVINGS OF DIET DRINK: MALES AND FEMALES COMBINED

Uses of table-top AS daily:	Diet drinks daily:		
	None	<2	⩾2
None	1·00* (1707, 3321)†	0·94 (314, 638)	1·21 (38, 60)
<3	1·02 (189, 367)	0·98 (212, 417)	1·26 (35, 54)
3–5	1·15 (80, 136)	0·76 (59, 146)	1·56 (20, 25)
⩾6	0·99 (18, 34)	1·53 (28, 34)	1·64 (7, 8)

* Relative risk of bladder cancer adjusted for age, race, sex
† (Number of cases, number of controls)

mated lifetime consumption (average daily dose multiplied by duration) showed no trends for males but a statistically significant, although erratic, positive trend for table-top AS among females.

In addition to the total study group, several subgroups of the study population were examined. First, we searched for effects detectable only in the absence of major bladder-cancer risk factors. Since males had three times the risk of females, and smokers had twice the risk of non-smokers, and those exposed to dye, leather, rubber, ink, or paint had 1·3 times the risk of those unexposed, we examined a group of female non-smokers unexposed to dye, rubber, leather, ink, or paint. Because we had few non-White women in this group, we further restricted the group to White women. These low-risk subjects had a crude risk of about 5 cases per 100 000 per year. Among these low-risk White women, we found a pattern of increased risk with increased levels of intake of AS (table IV). These findings were unaffected by adjustment for coffee-drinking, history of diabetes, geographic region, education, obesity, use of hair dyes, or history of urinary infections. A relation of risk to duration was not apparent in the total subgroup. However the low-risk women who consumed diet drinks or table-top sweeteners at least twice daily showed increased relative risk with longer duration of use. Low-risk White females who consumed table-top AS at least twice daily for ten years or more had 2·7 times the risk of non-users; those who drank at least two diet drinks daily for ten years or more had 3·0 times the risk.

TABLE IV—AVERAGE DAILY USE OF ARTIFICIAL SWEETENERS AMONG LOW-RISK* WHITE FEMALES

—	Table-top sweeteners			Diet drinks		
	Cases	Controls	RR†	Cases	Controls	RR†
Never used AS	130	402	1·0	130	402	1·0
Ever used table-top /diet drink	82	210	1·2 (χ^2=1·163; p=0·12)	71	219	1·1 (χ^2=0·387; p=0·35)
<1 use/serving	15	53	0·9	36	132	0·9
1–1·9 uses/servings	17	43	1·2	16	43	1·2
2–2·9 uses/servings	21	36	1·8	7	14	1·6
⩾3 uses/servings	22	38	1·8 (χ^2=2·630; p<0·01)	3	6	1·6 (χ^2=1·075; p=0·14)
⩾2 uses/servings for 5 yr	14	34	1·3	1	6	0·5
⩾2 uses/servings for 5–9 yr	13	22	1·8	3	7	1·4
⩾2 uses/servings for ⩾10 yr	16	18	2·7 (χ^2=3·240; p<0·01)	6	7	3·0 (χ^2=1·654; p<0·05)

* Never smoked cigarettes and never handled dye, rubber, leather, ink, or paint on any job; † adjusted for age. p values are based on one-tailed tests.

To search for potentiation of known bladder carcinogens, we examined a high-risk group composed of White men who smoked cigarettes most heavily (more than 40 per day) (table v). Within this group, consumers of diet drinks or table-top AS were at higher risk than those who never used AS. There were gradations in risk suggestive of dose-response for diet drinks, but not for table-top AS.

These findings were restricted to the heavy smokers; non-smoking men and those who smoked 40 or fewer cigarettes per day showed no significant trends in relative risk with increased use of either table-top AS or diet drinks. In fact, the non-smoking males showed a non-significant decreasing trend with increased daily use of diet drinks. Among the heaviest-smoking females (more than 20 cigarettes daily) the heavier users of table-top AS and diet drinks (⩾ 2 servings per day) also showed higher relative risks than those who never used AS. The trend in relative risk was significant for diet drinks. No consistent trends were seen for either form of AS among women who smoked 20 or fewer cigarettes daily.

TABLE V—AVERAGE DAILY CONSUMPTION OF TABLE-TOP AS AND DIET DRINKS AMONG WHITE MALES WHO SMOKED MORE THAN 40 CIGARETTES DAILY

—	Cases	Controls	R R*
Never used AS	104	167	1·00
Table-top AS:			
<1 use	12	15	1·28
1–1·9 uses	19	14	2·07
2–3·9 uses	16	13	1·96
4–5·9 uses	8	10	1·33
⩾6 uses	7	7	1·86
		(χ^2=2·220; p=0·01)	
Diet drinks:			
<1 serving	39	53	1·20
1–1·9 servings	14	19	1·20
2–2·9 servings	10	5	3·33
⩾3 servings	6	4	2·62
		(χ^2=2·339; p=0·01)	

* Adjusted for age. p values are based on one-tailed tests

Discussion

The data from this study do not provide support for earlier reports of a relative risk as high as 1·6 for men who used table-top AS. Thus, this study rules out a strong or moderate carcinogenic effect on the human bladder of artificial sweeteners as these have been used in the U.S.A. in the past. In the total study group, there was no evidence of increased risk to long-term users or to those first exposed decades ago.

However, an excess risk was seen among subjects who reported use of both table-top AS and diet drinks and heavy use of one of the forms (six or more daily uses of table-top AS, or two or more daily servings of diet

drinks). The relative risks were small by epidemiological standards, and did not show a consistent dose-response relationship. Further, the estimates for the heaviest users were based on small numbers of subjects.

Because of inconsistencies in the patterns observed, the elevations in risk require cautious interpretation and further analyses. Nonetheless, the findings give some cause for concern. First, it is not implausible that a carcinogenic effect might be seen only among the few subjects who were heavily exposed. Second, to the extent that levels of AS consumption have been rising, the future overall association between AS use and bladder cancer may be better approximated by the associations seen at higher doses than by the average past experience of the total group of subjects.

We examined a subgroup of women at low risk of bladder cancer because experiments with laboratory animals suggested that saccharin and cyclamates are weak carcinogens, whose effects may be more easily identified in persons unexposed to potent risk factors. Indeed, in the low-risk group there was evidence of an association with heavier consumption of diet drinks and table-top AS. It is noteworthy that a very recently reported study revealed no overall association between bladder cancer and artificial sweeteners, but did show an association among non-smoking females.[21] We were unable to determine whether the lack of association among low-risk males reflected their higher background risk compared with females or the role of chance in the positive association among females.

When we classified subjects according to their usual level of cigarette smoking, we found some associations between bladder cancer and AS among the heavier smokers. These elevated risks may reflect potentiation by AS of the carcinogenic effect of cigarette smoking, but further analyses of duration, total dose, and other factors are needed before an interpretation can be offered confidently.

While the positive associations observed in this study may reflect biological reality, other explanations are possible. Cases were identified and interviewed promptly, but 14% were too ill or had died. If these cases had histories of AS use greatly different from those of healthier cases, our estimates of relative risk would be biased. This would also happen if the cases or the controls who refused or were unlocated had histories greatly different from those of respondents. However, our response rates were high and were similar for cases and controls. Other biases could arise—for instance, if publicity had caused bladder-cancer patients to recall their AS use to a greater or lesser extent than other people, or if physicians were more likely to diagnose bladder cancer in an AS user. We think it unlikely that biases would have produced either the patterns of positive associations observed in various subgroups, which vary by extent of exposure and by other factors, or the overall lack of association with average AS use in the total study population. It is harder to exclude the possibility of chance. By chance alone, we could have missed a small but real elevation in risk associated with average past levels of use; and, by chance alone, we could have observed positive associations in subgroups of a study which, overall, showed no association between bladder cancer and AS use. On the basis of the confidence intervals, we doubt that chance played an important role in producing the overall lack of association.

The study did not assess the effects of exposure to AS in utero. Nor could it assess the long-term effects of certain newly established patterns of AS use, such as relatively heavy exposure begun in childhood. The analyses presented do not separate cyclamates (in use in the 1960s) from saccharin, but none of the associations seen were derived solely from exposures in the 1960s.

We conclude that past AS use has had a minimal effect, if any, on bladder cancer rates. We also conclude that the positive associations in this study do not by themselves establish a causal link between AS use and bladder cancer. However, we think it noteworthy that the pattern of positive associations is consistent with experimental data that suggest that artificial sweeteners are weakly carcinogenic when given alone and potentiating when given with other carcinogens.

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REFERENCES

1. Howe GR, Burch JD, Miller AB, et al. Artificial sweeteners and human bladder cancer. *Lancet* 1977; ii: 578–81.
2. Wynder EL, Stellman SK, Austin H. Saccharin usage and bladder cancer. Annual Report to NCI and Subcontract SHP-74-106C under NO1-CP-55666.
3. Wynder EL, Stellman SK. Saccharin usage and bladder cancer. *Science* 1980; **207**: 1214–16.
4. Jain MG, Morgan RW. Bladder cancer: smoking, beverages, and artificial sweeteners. *Can Med Assoc J* 1974; **111**: 1067–70.
5. Wynder EK, Goldsmith R. The epidemiology of bladder cancer: a second look. *Cancer* 1977; **40**: 1246–68.
6. Simon D, Yen S, Cole P. Coffee drinking and cancer of the lower urinary tract. *J Nat Cancer Inst* 1975; **54**: 587–91.
7. Kessler II, Clark JP. Saccharin, cyclamate and human bladder cancer. *JAMA* 1978; **240**: 349–55.
8. Arnold DL, Moodie CA, Grice HC, et al. Long-term toxicity of ortho-toluene sulfonamide and sodium saccharin in the rat: an interim report. Ottawa, Canada: National Health & Welfare Ministry, Health Protection Branch, Toxicology Research Division, 1977: 37.
9. U.S. Department of Health, Education and Welfare Food and Drug Administration. Histopathologic evaluation of tissues from rats following continuous dietary intake of sodium saccharin and calcium cyclamate for a maximum period of two years. Final Report (Project P-169-170). Washington, DC: U.S. Dept Health, Education, Welfare, Public Health Service, Food and Drug Administration, 1973.
10. Wisconsin Alumni Research Foundation. Long term saccharin feeding in rats: final report. Madison, Wisconsin: WARF, 1973.
11. Price JM, Biava CG, et al. Bladder tumors in rats fed cyclohexylamine or high doses of a mixture of cyclamate and saccharin. *Science* 1970; **167**: 1131–32.
12. Hicks RM, Chowaniec J. The importance of synergy between weak carcinogens in the induction of bladder cancer in experimental animals and human. *Cancer Res* 1977; **37**: 2943–49.
13. Cohen SM, Arai M, Jacobs JB, and Friedell GH. Promoting effect of saccharin and DL-tryptophan in urinary bladder carcinogenesis. *Cancer Res* 1979; **39**: 1207–17.
14. Waksberg J. Sampling methods for random digit dialing. *J Am Stat Assoc* 1978; **73**: 40–46.
15. MacMahon B, Pugh TF. Epidemiology principles and methods. Boston: Little, Brown, 1970.
16. Gart JJ. Point and interval estimation of the common odds ratio in the combination of 2×2 tables with fixed marginals. *Biometrika* 1970; **57**: 471–75.
17. Prentice R. Use of the logistic model in retrospective studies. *Biometrics* 1976; **32**: 599–606.
18. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Nat Cancer Inst* 1959; **22**: 719–48.
19. Mantel N. Chi-square tests with one degree of freedom; extension of the Mantel-Haenszel procedure. *J Am Stat Assoc* 1963; **58**: 690–700.
20. Morrison AS, Cole P. Epidemiology of bladder cancer. *Urol Clin N Am* 1976; **3**: 13–29.
21. Morrison AS, Buring JE. Artificial sweeteners and cancer of the lower urinary tract. *N Engl J Med* 1980; **302**: 537–41.